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Crayfish disease diagnostics - towards a Nordic standard

Nordic workshop 7.2. - 8.2.2007
Kuopio, Finland

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edited by
Satu Viljamaa-Dirks

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Kuvailulehti

Julkaisija	Elintarviketurvallisuusvirasto Evira, Tutkimus- ja analytiikkaosasto
Julkaisun nimi	Crayfish disease diagnostics - towards a Nordic standard
Tekijät	Satu Viljamaa-Dirks (ed.)
Tiivistelmä	Rapu on ollut jo kauan osa pohjoismaista kulttuuriperinnettä. Yli sata vuotta sitten Manner-Euroopasta levinnyt tuhoisa raputauti, rapurutto, hävitti kuitenkin suuren osan tuottavista jokirapukannoista. Monet rapuruton esiintymiseen ja diagnostiikkaan liittyvät kysymykset ovat vielä ratkaisematta vuosikymmeniä kestäneestä tutkimustyöstä huolimatta. Raputautien diagnoosimenetelmiä ja käynnissä olevia tutkimushankkeita käsiteltiin Kuopiossa järjestetyssä tapaamisessa, johon kutsuttiin edustajat pohjoismaisista raputauteja tutkivista laboratorioista. Kokous järjestettiin Elintarviketurvallisuusvirasto Eviran Kuopion yksikössä. Tutkijoita Suomesta, Ruotsista, Norjasta, Virosta ja Latviasta oli paikalla, samoin kuin OIE- rapuruton referenssilaboratorion asiantuntija Englannista. Tapaamisen ensimmäisenä päivänä käsiteltiin eri maiden rapukantoja, raputautitilannetta ja tautien tunnistusmenetelmiä. Toisena päivänä keskusteltiin tarkemmin eri diagnoosimenetelmistä sekä raputauteihin kohdistuvasta tutkimuksesta. Tapaaminen järjestettiin Pohjoismaiden Ministerineuvoston tuella.
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Resumé	Kräftan har sedan länge varit en del av den nordiska kulturtraditionen. Kräftpesten, som spred sig från Central-Europa för drygt hundra år sedan, förstörde dock en stor del av de produktiva flodkräft-stammarna. Trots intensiv forskning är flere frågor angående förekomst och diagnostik av kräftpest fortfarande olösta. För att evaluera pågående forskning och diagnostiska metoder i olika länder, ordnades ett möte i Kuopio mellan de olika nordiska länderna som undersöker kräftsjukdomar. Mötet ordnades i Livsmedelssäkerhetsverket Eviras enhet i Kuopio. Forskare från Finland, Sverige, Norge, Estland och Lettland var närvarande på mötet tillsammans med en representant för OIE-referenslaboratoriet för kräftpest i England. Under mötets första dag behandlades frågor angående de olika ländernas kräftstammar, kräftsjukdomssituationer och diagnostiska metoder. Den andra dagen behandlades de diagnostiska metoderna i detalj, samt pågående forskning i kräftsjukdomar. Mötet ordnades med finansiellt understöd av Nordiska Ministerrådet.
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Description

Publisher	Finnish Food Safety Authority Evira, Research Department
Title	Crayfish disease diagnostics - towards a Nordic standard
Authors	Satu Viljamaa-Dirks (ed.)
Abstract	Crayfish utilization is a long-standing Scandinavian tradition, which unfortunately has suffered a major drawback with the introduction of crayfish plague disease about a hundred years ago. In spite of intensive research many problems in management and diagnosis of this disease are not yet solved. To evaluate ongoing research and diagnostic methodology in different countries, representatives of diagnostic laboratories involved in crayfish disease diagnostics were invited to a workshop in Kuopio to discuss the problems with crayfish diseases. The workshop was held at the Kuopio unit of the Finnish Food Safety Authority Evira. Participants from Finland, Sweden, Norway, Estonia and Latvia were present, as well as the OIE expert from the reference laboratory for crayfish plague. In the programme of the first day the state of crayfish stocks, crayfish diseases and diagnostic methods used in each country were presented and discussed. During the second day diagnostic methods were discussed in detail, as well as research projects concerning crayfish diseases. The workshop has been made possible by way of a grant from the Nordic Council of Ministers
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Foreword

Utilization of crayfish is a long-standing Scandinavian tradition, which unfortunately has suffered a major drawback with the introduction of crayfish plague disease about a hundred years ago. In spite of intensive research many problems in the management and diagnosis of this disease are not yet solved. To evaluate ongoing research and diagnostic methodology in different countries, representatives of diagnostic laboratories involved in crayfish disease diagnostics were invited to a workshop in Kuopio to discuss the problems with crayfish disease. The workshop was held at the Kuopio Unit of the Finnish Food Safety Authority Evira. Participants from Finland, Sweden, Norway, Estonia and Latvia were present, all together 26 people.

In the first day's programme the state of crayfish stocks, crayfish diseases and the diagnostic methods used in each country were presented and discussed. Even with the most important disease, crayfish plague, there were significant differences in methodology in different laboratories. In the Baltic countries the ability to make a confirmed diagnosis was non-existent. This was in sharp contrast to Norway, where a modern method based on molecular techniques has been developed. In Central Europe also, problems were encountered, with only three countries having been able to confirm the presence of crayfish plague. The status of crayfish plague as an OIE-listed disease warranted a separate discussion because of the problems concerning the existence of non-native, disease carrying crayfish species in most of the European countries.

During the second day methods were discussed in detail, as well as research projects concerning crayfish diseases. In a round-table discussion it was agreed to apply for funding for a more continuous network project, which aims to help all involved laboratories to develop reliable methods for crayfish disease studies. This should result in a better understanding of crayfish diseases, leading to better tools for management of crayfish stocks.

The Nordic and Baltic countries are among the few countries in Europe that still have considerable stocks of native European crayfish species. Protecting these stocks is not only important economically and for species conservation, but also for maintaining a part of Scandinavian heritage.

This workshop has been made possible by way of a grant from the Nordic Council of Ministers. On behalf of the organization and all the participants, I want to thank the Council for making this start of our fruitful co-operation possible. A warm atmosphere was experienced in Kuopio, in spite of the freezing cold weather of minus 35 degrees Celsius during the two days.

Satu Viljamaa-Dirks
Local organizer
Evira, Kuopio

Contents

Introduction

Satu Viljamaa-Dirks
Finnish Food Safety Authority, Kuopio

Crayfish is part of the cultural heritage of the Scandinavian countries, as well as a commercially important species. Successful management of crayfish stocks requires reliable diagnosis of crayfish diseases, especially crayfish plague, which has been the main limiting factor in the development of crayfish fisheries and farming. As the main producers of crayfish in Europe, the Nordic countries have the most needs and best opportunity for further development of crayfish disease diagnostics. Nordic co-operation and standardisation of methods are lacking at the moment

The most serious disease of European freshwater crayfish, crayfish plague, is caused by an oomycete fungal-like organism *Aphanomyces astaci.* The problems in the isolation of the fungus from diseased crayfish are well illustrated by the period of several decades before the acknowledgement of the causative agent (Schikora 1903, Nybelin 1934). The crayfish plague arrived to Southern Europe in the 19th century, reached Finland in 1893, Sweden in 1907 and finally Norway in 1971.The populations of the noble crayfish *Astacus astacus* were decimated everywhere where the plague appeared. The infection apparently originates from North America, having been found in clinically healthy North American species of crayfish. The North American species Pacifastacus leniusculus, the signal crayfish, was introduced to Scandinavia about 50 years ago to replace the plague-stricken populations of noble crayfish. The signal crayfish is to be found in most of the Scandinavian and Baltic countries, excluding Norway and Estonia (Mannonen and Halonen 2000). In its natural host, the crayfish plague lives in a parasitic balance with the host. The host can restrict the growth of the parasite by an effective defence mechanism, based mainly on a quick response to a germinating cyst and encapsulation through melanisation. In susceptible crayfish species the defence mechanism is genetically different, the first reaction to the penetrating hyphae is delayed and often the crayfish will not succeed in limiting the growth of the pathogen (Bangyeekhun 2002). This leads to an acute crayfish plague, the hyphae protruding into other tissues of the crayfish and finally causing the death of the animal. When the immune system of resistant species is compromised, they can develop an acute disease form as well. This has been shown experimentally (Persson et al. 1987) and in practice, where significant mortality of cultured signal crayfish by crayfish plague is often recorded (Finnish Food Safety Authority, unpublished). Crayfish plague spreads effectively through spores, which can live outside the host for several weeks. Spores can be transported with traps, boats, fishing gear and even fish. Transporting infected crayfish is an efficient way of spreading the plague, which remains viable several days even in dead individuals (Oidtmann et al 2002). Theoretically also other animals like birds could transmit the disease, but this has never been shown.

Wherever infected resistant crayfish species are present, the infection must be considered endemic. There is some discrepancy concerning the temporary nature of the infection in susceptible crayfish populations. Traditionally,

total mortality of affected stocks has been expected, based on laboratory experiments (Alderman et al. 1987). There is, however, clinical evidence that it might not always be the case. In complicated waterways like the lake systems in Finland, a phenomenon called the chronic plague has been long known. In that kind of waterways, it has never been possible to re-establish a permanent noble crayfish population, because of the recurrent epizootics of crayfish plague. The prevailing theory explained the mortalities by the existence of several subpopulations, where the plague could slowly migrate from one subpopulation to another (Westman 1991). This could be one mechanism for the survival of the infection. Fürst (1995) has studied the history of crayfish plague in Swedish lakes and came to the conclusion that the most probable explanation for the failure of the noble crayfish populations to re-establish themselves, was the chronic state of *A. astaci* infection in the weak crayfish populations. Because of improved detection techniques, it has now been possible to verify the presence of a permanent infection in such a population, where the infection apparently does not lead to an acute outbreak (Satu Viljamaa-Dirks, unpublished). In the long history of the plague, a cyclic appearance of the acute outbreaks has been noted in parts of the infected lakes (Nylund and Westman 1995). It could be hypothesized that a low level of infection would allow the population of susceptible crayfish to increase to the point where it is dense enough to suffer a new acute outbreak.

The long history of the spread of crayfish plague in Europe mainly concerns wild stocks and is well recorded by Alderman (1997) among others. The prevalence in carrier crayfish species has not been screened, but experience suggests that even pathogen-free signal crayfish specimens used for stockings contract the disease sooner or later. Some acute outbreaks have been recorded in feral signal crayfish in Finland and Sweden, but the reason for these is not clear (Mannonen and Halonen 2000). There is no exact information available concerning crayfish mortality and the prevalence of crayfish plague. The number of noble crayfish population mortalities in Finland is estimated to be 10-20 annually (Mannonen and Halonen 2000). In many cases the cause of the mortalities cannot be examined because of the lack of sample material. This is especially true with mortalities occurring during the winter period, when the lakes are covered with ice for several months. The majority of mortalities are suspected to be due to crayfish plague infection, other reasons like environmental stress being less common (Nylund and Westman 1995). Crayfish fisheries are an important form of inland fisheries but have been severely affected in most of the countries contaminated by the crayfish plague. In Finland the catch in the best years was about 15-20 million noble crayfish. Lately the catch has been 0.7-4 million noble crayfish, and 0.5 -2 million signal crayfish (Wildlife and Fisheries Institute, annual reports 1986 - 2004), the catch of signal crayfish is growing fast. However, crayfish still represents the largest economic value of a single species in inland fisheries (Nylund and Westman 1995). In Sweden, about 90% of the original stocks of noble crayfish have been estimated to have disappeared because of the plague (Fürst 1995). The signal crayfish, once introduced to improve the fisheries, has managed to establish itself in most parts of what were once habitats of native crayfish species. It has become a commercially important species in Sweden as well as in Finland. At the same time, by spreading to new areas it keeps on restricting the living space of native crayfish. Mixed populations have been recorded occasionally, but the native species will eventually disappear through direct competition and/or the permanent presence of crayfish plague.

Crayfish plague carriers in the waterways also make it difficult to start economically interesting crayfish aquaculture because of the high risk of losing the entire stock. Crayfish farming has been developing since the 1960's especially in Sweden, Finland and Germany mainly by culturing noble crayfish as well as signal crayfish. The culture can be intensive, semi-intensive or extensive as naturally feeding populations in ponds. Farms produce juveniles for stockings and/or table-size adults. The biggest restriction for the culture of the noble crayfish or other susceptible species is the lack of plague-free waterways, since the infection will mostly lead to a total mortality of the stock. Mortality in noble crayfish farms due to crayfish plague has been reported (Oidtmann et al 1999a), but earlier difficulties in diagnosing *A. astaci* probably restricted the number of published reports. In most countries, establishment of a crayfish farm has not been

restricted in the same way as introductions to the wild. This has led to localisation of signal crayfish farms even in the areas where considerable stocks of susceptible species are present. It is very likely that infection spreads from the farm to the surroundings by escapees or infective spores. To enhance the crayfish fishery, a much used method is stocking with feral crayfish from elsewhere or crayfish cultured for the purpose. Certainly, if infected signal crayfish are introduced into natural waters, transfer of the infection can be expected to happen with wild as well as cultured stocks (Alderman et al 1990). The lack of a suitable screening method has prevented certification for freedom from disease.

The development of molecular methods has shed new light on the epidemiology of the crayfish plague. By using the RAPD-PCR method four different genetic types have been recognised (Huang et al 1994, Dieguez-Uribeondo et al 1995). The Astacus strains (As-type) have been isolated from noble crayfish before the large scale introductions of signal crayfish to Europe, and from narrow-clawed crayfish from Turkey. Pacifastacus strains group I (PsI) have been isolated from signal crayfish and noble crayfish after the introductions of signal crayfish. There are two strains that were different from these two types, one from signal crayfish of Canadian origin (Pacifastacus strain group II, PsII), and one from the red swamp crayfish (Procambarus strain, Pc), the latter showing some physiological differences as well. By genotyping it has been possible to show the connection of crayfish plague epizootics with the introductions of alien crayfish species (Lilley et al 1997, Vennerström et al. 1998, Oidtmann et al. 1999a). In the areas where resistant crayfish species have become naturalised, crayfish plague epizootics in susceptible species seem to be caused by the PsI type (Royo et al. 2004). In Finland, where the introductions of signal crayfish have mainly been restricted to the southern part of the country, the majority of the isolates from the northern and eastern parts are still of the type As (Viljamaa-Dirks and Heinikainen 2003). Epidemiological evidence suggests some difference in the virulence between the types As and PsI, but experimental work has not been published so far (Satu Viljamaa-Dirks, unpublished).

The detection of *A. astaci* has been very complicated. The main disease outbreaks have been in wild stocks, and the first obstacle is to recognise the problem in time, so that suitable sample material can be found. If symptomatic or highly infected animals are found, microscopic study will often reveal hyphal growth in the cuticle. There is, however, no definitive diagnosis to be reached by microscopy alone, since several other water moulds have been identified in the cuticle of the crayfish (Oidtmann et al. 1999b), nor does negative microscopy exclude the possibility of infection (Viljamaa-Dirks and Heinikainen 2006). The infection can be verified by isolating the agent by placing the infected parts on a suitable growth media. Since sexual propagation has not been recorded, identification to species level by morphological features is not possible and until recently had to be performed by infecting susceptible crayfish with artificially produced spores (Cerenius et al. 1988). Isolation is often complicated by abundant growth of contaminants, and demands some experience. A molecular method to aid the rapid diagnosis has been published by Oidtmann et al. (2004). Although very useful for quick detection and thus an aid for preventing unnecessary spread of the disease, the method cannot separate between the different genomic types of the crayfish plague organism, making it less suitable for epidemiological studies.

The confirmative diagnosis of crayfish plague has been considered possible by the culture method only in acute cases of the plague in moribund or newly dead individuals (Alderman and Polglase 1986). In an epizootic occurring in natural waters, these kinds of individuals are rarely caught, not least because the crayfish fishing season in Northern countries is restricted to a period of a few months and the follow-up of the populations outside this period is only coincidental. It has been long known that the combination of infection doses and temperature has a significant effect on the development of pathology in the host animal (Alderman et al. 1987). At the height of mortality, most individuals are heavily infected offering a good chance for successful isolation. Samples from mass mortalities are usually received towards the end of the epizootic, when infection pressure has already diminished. When

performing microscopic examination, small foci of infection are easily overlooked. By using the commonly used diagnostic procedure part of the cases are missed because of inaccurate microscopy (Viljamaa-Dirks and Heinikainen 2006).

There is no doubt of the devastating effect of the crayfish plague on wild as well as cultured European crayfish species. The infection is also a risk factor in the culture of resistant species. The long term effect of the infection in feral signal crayfish has to be clarified, since some evidence exists that the disease can affect even them. The present suspicion of crayfish plague being able to reside also in the noble crayfish populations without immediately causing a noticeable outbreak creates a new situation from the management point of view. It is essential to develop sensitive detection methods that can discover a low level of infection, to be able to recognise the infected populations of all crayfish species. The reservoirs of *A. astaci* should be found by systematic screening of the populations at least in the areas where it has not yet become endemic.

Several parasites are recognised to infect crayfish species, but the clinical significance of them remains unresolved. Other fungal and bacterial diseases, as well as viral disease, need to be explored as well. Besides the crayfish plague diagnostics, co-operation between laboratories that handle crayfish samples could greatly enhance the experience and further development of the understanding of crayfish diseases.

A meeting with representation from of all of the Nordic and Baltic countries that have an interest in crayfish was considered necessary to evaluate the present situation and to make further plans for co-ordinated method standardisation and research. The aim is to achieve a common diagnostic level in all of the Nordic countries, and thus aid in developing the management of crayfish stocks.

To create a network of crayfish disease diagnostic laboratories, a two-day seminar was organised in Kuopio, Finland, by the Kuopio Research Unit of the Finnish Food Safety Authority Evira. Crayfish plague diagnostics in Finland are performed at this laboratory, and research has been done to improve the diagnostics and understanding of crayfish plague. Some contacts had been created earlier between Finland and Sweden, Norway, Estonia and Russia.

The workshop was funded by a grant from the Nordic Council of Ministers, Nordic Working Group on Fisheries Research. Finnish Food Safety Authority Evira offered the meeting facilities.

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Programme

The two-day workshop was divided into two seminars. During the first day, the crayfish stocks, the diagnostic possibilities and the disease situation in each of the countries was discussed under the heading “Crayfish diseases - confirmation and consequences”. At the end of the day, a short discussion took place, concerning the World Organisation for Animal Health (OIE) classification of crayfish plague as an A-list disease. Problems arise with reporting the epizootics, because most of the European countries have introduced populations of carrier species. During the second day recent research results, as well as the nature of ongoing and future research projects were discussed under the heading “Research in crayfish disease diagnostics”. The emphasis lay in the modern molecular techniques available at the moment. Future co-operation was discussed as well. There was a general agreement about the need to form a co-operative network for disease diagnostic and research matters. A preliminary project form was discussed, and the Norwegian researcher Dr. Trude Vrålstad was appointed as the contact person in drafting an application for financial support of the necessary expenses of such network.

The programme is presented in annex I.

Participants

Nordic and Baltic laboratories, known to be involved with crayfish disease diagnostics, were invited to participate in the meeting. Representatives from laboratories in Finland, Sweden, Norway, Estonia and Latvia attended. Representatives from Lithuania and Russia were not able to attend, but were interested in the results of the meeting and future co-operation. The expert of the OIE reference laboratory in England was invited as a speaker. Several researchers from Finland, Sweden and Estonia were present as well.

The list of participants is presented in annex II.

Crayfish in Finland

Timo Ruokonen, Markku Pursiainen and Riitta Savolainen
Finnish Game and Fisheries Research Institute

Finnish Game and Fisheries Research Institute runs the "Crayfish Research Program 2005-2012" The program consists of several research and development projects. The aim of the program is to study the biology and ecology of noble (*Astacus astacus*) and signal crayfish (*Pacifastacus leniusculus*) together with the socio-economy of crayfish production. Research-generated knowledge is needed for understanding the influence of the rapid increase in signal crayfish production and for maintaining the noble crayfish stocks. The main outline of the program is to identify the differences in the distribution and habitat requirements of the two crayfish species, and to estimate the crayfish production and catch development and the consequences of the obvious changes.

In 2006 the program completed the first crayfish survey. The survey consists of data collected from the crayfish stockings' register during the last few years, crayfish plaque, and the development of crayfish capture with the crayfish farming statistics. The aim of the annually updated crayfish surveys is to create a general picture of the potentials and limitations of the crayfish stock, catch and management development with the economy combined to these.

The noble crayfish introductions and stockings have a long history in Finland, and the noble crayfish distribution area has multiplied compared to the situation 100 years ago, the time when the plaque outbreak collapsed the production. The signal crayfish was brought to Finland for the first time in 1968. The data from the crayfish stockings in 1989–2004 was attained from the introduction registers maintained by the local Employment and Economic Development Centers. During that time 1,91 million noble crayfish was introduced in a total number of 716 lakes and 350 rivers. The introductions of signal crayfish, and thereby the distribution, are concentrated mainly in southern Finland. During 1989-2004 about 1,74 million signal crayfish was introduced in 277 lakes and 75 rivers. The volume of crayfish releases has decreased from 400 000 in the top years in the 1990's to approximately 100 000 individuals. Noble crayfish has been introduced mainly in smallish lakes, smaller than 500 hectares, whereas signal crayfish introductions were concentrated to larger lakes.

Crayfish catch statistics have been made since 1984 every second year in the Finnish Game and Fisheries Research Institute in connection with the Recreational Fishing Statistics. The statistics anyhow contain insecurities due to the small number of crayfish catchers and the large variations of the catch per fisherman. In spite of this, the general picture of the importance of the crayfish fisheries is obvious.

The annual catch in Finland fluctuated in 1986-2004 from approximately 1,6 million to over 4,8 million noble and/or signal crayfish. No clear development tendency can be outlined. The mean annual catch has been about 3 million crayfish. The two crayfish species has been separately distinguished only twice. The noble crayfish catch in 2001 was estimated to 1,7 million and the signal crayfish to 0,7 million individuals. In 2004 the abundance index for the species seems to have turned the opposite.

The trend is expected, but the exact numbers uncertain. Until the mid 1990's the catch was almost exclusively noble crayfish. The crayfish catching is carried on in approximately 20 000-30 000 households.

The crayfish farming began in the 1980's, and at the same time increased the introductions and stockings of noble and signal crayfish. The farming of crayfish is concentrated in southern Finland. Stocking material was the main product in the 1980's and 1990's. The demand for stocking material has decreased and some farmers have moved on to the production of crayfish for food. In 1997 some 140 producer were listed in the aquaculture register, but in 2005 only 111, of which 75 operating. Farming is mainly a part-time business.

In 1993 the number of juvenile crayfish produced was some 390 000 individuals. From this the production has gradually decreased to a level of 80 000-120 000 individuals. The signal crayfish quota of the production has been 60-95 %. Crayfish aquaculture production for food was estimated to 56 000–83 000 individuals in 2002, which corresponds to 2 500–3 700 kg. In 2005 the production was estimated to 52 000 ± 23 000 individuals. Both years about three quarters of the production was signal crayfish.

Crayfish disease in Finland

Satu Viljamaa-Dirks
Finnish Food Safety Authority Evira

The main disease problem in Finnish crayfish populations is crayfish plague. Other identified specific infections are caused by the parasites *Psorospermium haeckeli* and *Thelohania contejeani*. Infection with baculovirus has been recorded in some crayfish populations, without causing clinical signs. During the years 2000-2005 278 batches of noble crayfish and 39 batches of signal crayfish were studied for pathology. Crayfish plague was identified in 66 noble crayfish samples and 12 signal crayfish samples. *Psorospermium haeckeli* was found in 58 noble crayfish samples, but not all batches were studied in order to find it. This parasite was not recorded in the signal crayfish. Thelohania contejeani was found in 4 cases in noble crayfish. In 32 batches of noble crayfish and 4 batches of signal crayfish miscellaneous pathological conditions were recorded, mostly for unidentified reasons.

Crayfish plague has attacked nearly all main noble crayfish populations during the more than hundred years of existence in Finland. It has been possible to identify the genotypes of the agent in many cases. Both the original genotype (Astacus type), that was introduced first to Europe, and the later introduced genotype (Pacifastacus strain I type) carried by signal crayfish, are found in Finland. Not surprisingly, most isolations of signal crayfish type strains are made in southern Finland, in and next to the areas where signal crayfish are stocked in Finnish natural waters. There are some anamnestic differences connected with the isolations of the two genotypes. It seems that signal crayfish type strains are more often connected to episodes of acute mortality, while the Astacus type is more often found in weak populations and cage experiments, often engaged with repopulation programmes.

The diagnosis of crayfish plague has been based on the cultivation and identification of *Aphanomyces astaci*. Some minor changes in the cultivation protocol improved the yield of positive samples significantly. It became also evident, that a plague infection did not necessarily lead to the expected total mortality in a short time, even during the warm summer months. It seemed that slow-growing strains of Astacus type especially could exist longer in a weak population. In a follow-up study of a small Finnish lake, isolations of this genotype were made several years after the initial outbreak. In another case, the mortality was followed in one cage experiment throughout one year, and isolations were made at the beginning as well as at the end of that period. The apparent existence of low level infections by crayfish plague has consequences for present management practices. It has been usual to start a repopulation programme rather soon after a crayfish plague epizootic. If there are infected survivors, they will infect the newly stocked crayfish and the reintroduction fails – a situation that is not uncommon in connection with repopulation programmes. It can even be possible to move the crayfish plague agent from one water body to another with seemingly healthy but infected carriers- a situation that has been considered impossible. It is of vital importance, that the possibility of noble crayfish being a carrier of crayfish plague is taken into account, and sensitive laboratory methods to detect the carrier status are needed.

Crayfish and crayfish disease in Estonia

Tiit Paaver, Margo Hurt, Katrin Kaldre, Kuldar Kõiv
Department of aquaculture Estonian University of Life Sciences ,Tartu, Estonia

Current situation of crayfish stocks in Estonia

Noble crayfish *Astacus astacus* (L.) is the only freshwater decapod species in Estonia. No introduced alien species incl. signal crayfish. The data about crayfish situation in Estonia are mainly based on test fishing with traps. According to data from 1993-2006 noble crayfish is present in 256 lakes or streams. More than half of them (53%) are sparse – CPUE below 1. In one third of crayfish sites is moderate population. Only 14% of crayfish populations are dense or very dense (CPUE over 4). The best crayfish region is the island Saaremaa, which is a crayfish plaque free refugium. In 2004-2006 the reported catch of fishermen was stable remaining between 7300-7600 specimens.

Crayfish plague in Estonia

In Estonia as everywhere, the basic disease problems are caused by crayfish plague. In the end of 19th and in the first half of 20th century several waves of crayfish plaque wiped out nearly all crayfish stocks on mainland of Estonia. Only the natural crayfish stocks on the islands of Saaremaa, Muhumaa and Hiiumaa have not been affected by crayfish mass mortalities.

Recently a few mortalities of crayfish with plague like symptoms have been detected but never correctly diagnosed. As the presence of plague agent (*Aphanomyces astaci*) was not correctly analysed we can consider those cases as plague-like mortalities. During last 10 years in 6-7 water bodies and in one crayfish farm plague-like mortalities were reported. Most of those water bodies contained a good crayfish stock. Besides, some cases of disappearing sparse crayfish population were reported, but these could not be considered as plaque-like mortalities. The problem of Estonia is a lack of possibilities for correct diagnosis of crayfish plague.

Other crayfish diseases in Estonia

Burn spot disease is common in Estonia. It is very probable, that there are many different pathogens causing these external symptoms. In Estonia the typical burn wound like damages with orange zone around damage are rare. Typical form is just barely visible dark brown, almost black melanized area on carapace. After boiling it is especially clearly visible.

Collecting our data of porcelain disease is based on observing the presence of visible symptom (opaque white muscle). Any microscopic study has not been done. The porcelain disease is very common in Estonia. The percentage of infected specimen is usually not more than 1-2%.

In 1993-1997 *Psorospermium haeckeli* was found in 12 out of 21 lakes when analyzing a sample of 3-5 crayfish from each lake. In 2004 the parasite was not found in 6 populations located near to each other.

According the data of Järvekülg (1958) 3 species of *Branchiobdella* are present in Estonia. Recent (1993-2006) information is mostly based on estimating abundance of shell parasites *B. parasita* and *B. pentodonta* as the species were not identified. Almost in all crayfish populations

the shell parasites are present, but the quantity is often very various in different water-bodies. Sporadically studied and more pathogenic gill parasite *B. astaci* is present in minor part of crayfish populations.

The influence of mentioned diseases (except plague) and parasites on crayfish stock is not estimated but it seems to be not very serious.

In 2005 and especially 2006 strange mortalities occurred in ponds of a crayfish farm. Many crayfish died during moulting. Some of the moulted crayfish had damages - lesions or pale areas on new shell, which were not visible on old shell. Many crayfish died and first symptom was opening a gap between carapace and tail and clearly visible white band of meat in this region (not moulting).

Diseases in crayfish in Europe

Birgit Oidtmann
Cefas, Weymouth Laboratory, Weymouth Dorset, UK

Crayfish plague dominates among the crayfish diseases as the disease of highest importance due to its high virulence. Characteristics (macromorphology, histology, epidemiology) of the disease were presented.

A disease occasionally seen in crayfish kept in suboptimal conditions, and therefore associated with a suppression of the immune system, is midgut disease, linked with an infection with *Citrobacter freundii*. Among the parasitic diseases, porcelain disease, caused by *Thelohania contejeani*, is usually lethal. A PCR method has been developed to detect infection. Prevalence within a population seems to vary greatly. Several species of Branchiobdellids colonise crayfish, most without having much detrimental affect on the host. However, some species are thought to reduce the hatch-rate, due to colonising the eggs. Knowledge on the impact of *Psorospermium haeckeli*, a parasite mainly infecting connective tissue, is currently very limited. It is thought to reduce the efficiency of the immune system and therefore make the crayfish more susceptible to other infectious agents.

Further diseases presented were a number of fungal infections, infestation with Epibionts, and a summary of viral infections.

Diagnostic methods for crayfish plague across Europe

Birgit Oidtmann
Cefas, Weymouth Laboratory, Weymouth Dorset, UK

National reference laboratories for aquatic animal diseases across Europe were contacted to find out if they were investigating crayfish disease outbreaks and if yes, if they had methods in place to diagnose crayfish plague. Scandinavian countries were not included in the study since the methods of diagnosis used in those countries were going to be presented at the meeting.

Replies were received from 13 countries. Nine out of those 13 countries do investigate crayfish mortalities; eight investigate for crayfish plague. The methods used to diagnose crayfish plague were culture (4 countries), PCR (1 country) or both PCR and culture (2 countries). In those countries, where culture was the only method used, crayfish plague has never successfully been confirmed, whereas in those countries using either PCR or both PCR and culture, it had.

The results suggested that culture of *Aphanomyces astaci* from infected specimen requires experience, whereas using PCR might be a method requiring less experience and potentially more suitable in laboratories were specialised staff is not available.

Crayfish plague in Norway – outbreaks and diagnosis

Trude Vrålstad, National Veterinary Institute (NVI), Oslo, Norway

In Norway, crayfish plague is classified as a group A disease, and noble crayfish (*Astacus astacus*) is included as an endangered species in the national red list. The history of crayfish plague in Norway is short compared to Finland and Sweden. The first mass mortality of noble crayfish was observed in the river Vrangselva in Norway in 1971, close to the Swedish boarder. Crayfish plague was suspected based on the disease history and observation of oomycete hyphae in the crayfish cuticle. However, attempts to isolate the agent in pure culture failed, and the diagnosis could not be verified. The next suspected outbreak was in the Glomma watercourse in 1987. Two years later, the Halden watercourse was infected. In the latter case, the infection had most likely been transferred from the lake Stora Le, where crayfish plague had been diagnosed on the Swedish side of the lake. Both in the Glomma and the Halden watercourses, mass mortalities were observed and direct microscopy revealed typical oomycete hyphal infections in the soft crayfish cuticle. Again, the diagnosis could not be verified as all attempts to cultivate the agent failed. In 1998 mass mortality of noble crayfish was observed in the river Lysakerelva, close to Oslo city. As in the previous cases, all attempts to cultivate the agent failed. Attempts to re-establish the noble crayfish populations of the Glomma and the Halden watercourses were done in 1989 and 1995, respectively. The populations were growing reasonably well for many years. However, in 2003, the re-established population in Glomma had suddenly disappeared without leaving any traces. In 2004, cage experiments with living control crayfish were established in Glomma, and within a few months mortality was observed. Again, crayfish plague was suspected, but could not be verified.

At this stage, the presence and importance of crayfish plague in Norway was questioned. Crayfish plague as the common reason to crayfish mortality could be overestimated, and other factors or agents could have been overlooked. At the same time, it was unquestionable that the standard OiE method for diagnosing crayfish plague had serious limitations. In many cases, crayfish submitted to the Veterinary Institute (Norway) for analysis are dead with various degree of degradation, while the standard method that relies on isolation in pure culture requires optimal conditions and living crayfish. In 2005, the Veterinary Institute decided to implement existing or develop new molecular methods for specific detection of the agent directly from crayfish tissues. The *A. astaci* specific PCR described by Oidtmann et al (2004) was considered, but not routinely used since detection of a single PCR product alone may not be sufficient for a confirmative detection of *A. astaci*. It was decided to solve this by confirmative sequencing. For this purpose, we designed another forward primer that in combination with the Oidtmann primer 640 yielded a 520 bp PCR product. In 2005, *A. astaci* was successfully detected for the first time in Norway based in this PCR assay followed by sequencing that in all cases confirmed a 100% species specific *A. astaci* sequence. In total, there were 14 positive cases

of crayfish plague from the Glomma and Halden watercourses in 2005. It was further developed a highly specific and sensitive real-time PCR assay. This method was tested on the cases from 2005, and was also used to test ethanol-fixed historical samples. Real-time analyses of the historical samples confirmed that *A. astaci* had been the causative agent of crayfish mass mortality in Norway in the period from 1971-2004. Presently, the *A. astaci* specific real-time PCR assay is used as the Norwegian "standard" for the diagnosis of crayfish plague. However, direct microscopy and disease history is as far as possible also taken into account.

A short overview about crayfish in Sweden

Eva Jansson and Thorbjörn Hongslo
The National Veterinary Institute, Uppsala Sweden

Crayfish and fishing of crayfish have a long tradition in Sweden. Already 1878 came the first law that restricted crayfish fishing in June-July. There was a good supply of the noble crayfish in lakes and small rivers at the end of the 19th century and crayfish was an important income for many farmers especially when the export to other countries in Europe increased. The high demand of crayfish was covered with import of living crayfish. The dumping of diseased and dead imported crayfish in the lake Mälaren just outside Stockholm 1907 resulted in the introduction of the crayfish plague in to Sweden. The infection got an epidemic spread possibly through human transmission but also through birds and other animals and 1960 was 95% of the former noble crayfish waters classified as infected with the plague. The reintroduction of the noble crayfish in plague infected waters failed and therefore started the import of the signal crayfish from United States. This introduction was successful and signal crayfish is today the major crayfish species in Swedish lakes and river systems. Today is the spread of the crayfish plague mainly due to uncontrolled release of signal crayfish. The noble crayfish is on the "red list" of species well worth to protect and signal crayfish is not allowed to be released into Swedish waters due to the risks for transmission of the crayfish plague.

The Swedish Board of Fisheries initiated 1989 requirements of a health control of crayfish before movement of crayfish to a new water system and allows yearly resources for investigations of unexpected mortalities. The National Veterinary Institute is since 2002 responsible for these investigations. Diagnosis of crayfish plague is in Sweden based on microscopic investigations of shell and soft tissue from infected areas and cultivation from these areas for isolation of *Aphanomyces astaci* on special agar. Initial studies with use of PCR have been performed at our laboratory. Preliminary results demonstrated that freezing of shell and tissue material in -20°C before extraction of DNA was more efficient compared with grounding in liquid nitrogen or bead-beating in a Tissue lyser. During 2006 has the microscopic investigations and/or in combination with culture also been compared with real-time PCR (Trude Vrålstad, National Veterinary Institute, Oslo). A positive diagnose by microscopy/cultivation was verified by real-time PCR in four out of six cases but further two positive cases were diagnosed by real-time PCR. At the end of 2005 was 23 water systems classified as infected with the crayfish plague (The Swedish Board of Fisheries, Fig 1). Signal crayfish was sent in to our laboratory from two lakes and stream systems due to acute mortality during 2006. Acute crayfish plague was regarded as causing the mortality in one of these cases. Iron precipitation on the gills in combination with gill parasites was assumed to be the reason for mortalities in the second case.

There are today one farm rearing signal crayfish in Sweden and 14 farms rearing the noble crayfish. These farms are controlled through the Swedish Fish Health Control Programme and samples are sent in each second year for investigations of the parasites *Thelohania sp* and *Psorospermium haeckeli* (25 crayfish from each farm). Samples are also collected from natural waters. There is a higher frequency of *Thelohania* and *P.haeckeli* detected in nobel crayfish compared with signal crayfish (Table 1).

Table 1. Diagnostics of Psorospermium haeckeli and Thelohania spp in Swedish crayfish farms and natural waters 1990 - 2006 (Ref. Ulf-Peter Wichardt, The Swedish Fish Health Control Programme)

	Noble crayfish infected sites/ total sites investigated	Signal crayfish infected sites/ total sites investigated
Psorospermium sp.	11/44	2/63
Thelohania spp	2/44	0/63
Psorospermium sp. and Thelohania spp	3/44	0/63

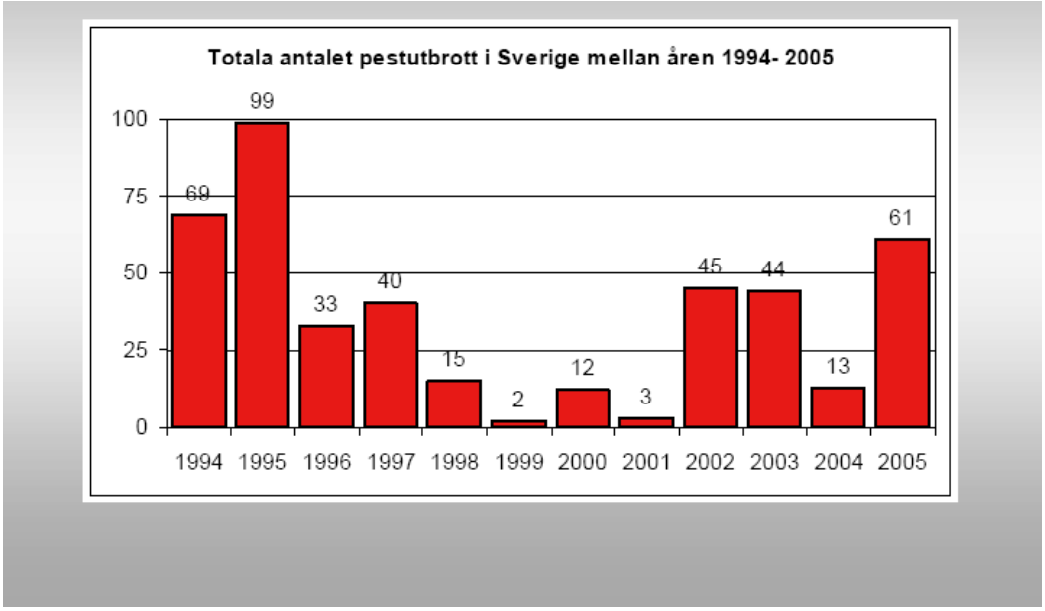

Figure 1. *Total number of acute crayfish plague in Sweden during 1994-2005 (Ref. Lennart Edsman, The Swedish Board of Fisheries)*

Crayfish in Latvia

Antra Skutela and Rita Granta
National Diagnostic Centre of Food and Veterinary Service, Latvia

There are four crayfish species in the natural water bodies of Latvia. Only one – noble crayfish (*Astacus astacus*) is native species, the other are introduced from other Europe and United States of America. These include slender clawed crayfish (*Astacus leptodactylus*), signal crayfish (*Pacifastacus leniusculus*) and also spinycheek crayfish (*Orconectes limosus*), which can be found in river Lielupe.

Whole noble crayfish aquaculture cycle farming (from rearing to production) is carried out only in five farms: SIA "Astacus", SIA "Vēži", SIA "Vlakon", farm of Mr. J. Krūmiņš in the region of Alūksne and farm of Mr. J. Kuzma in the region of Ludza. Noble crayfish stock production is done in about 15 farms. In aquaculture obtained young crayfish are used in renewal of crayfish population in Latvian waters. In 2006 almost 19'000 second summer crayfish were let in lakes and rivers of Latvia. The total production of crayfish is about 6 tonnes per year.

There are several crayfish diseases in Latvia. Respectively:

- burn spot disease, which is caused by fungi and/or bacteria. The disease is characterized by progressive erosion of the exoskeleton and is fatal when large areas of the exoskeleton have been eroded.

- microsporidiosis or porcelain tail disease.

The data about these two diseases is gathered as clinical observations provided from farmers because there are not any surveillance programmes on these diseases. And also Latvia is not free of crayfish plague, which is caused by the oomycete *Aphanomyces astaci*. There was an outbreak probably of crayfish plague in late 1960, but it was never confirmed in laboratory. In the late years there has not been any positive crayfish plague case as seen in the table:

The crayfish plague diagnostic was carried out following the guidelines from Manual of Diagnostic Tests for Aquatic Animals 2003, Chapter 4.1.7.1.2. Crayfish plague *(Aphanomyces astaci)*.

Year	Number of crayfish mortality/ disease cases submitted for investigation to our laboratory	Number of these investigated for crayfish plague	Cases diagnosed as crayfish plague (Positive
2006	-	-	-
2005	4	4	-
2005	7	7	-

Detection of *Aphanomyces astaci* in susceptible crayfish by PCR and Detection of *Aphanomyces astaci* in North American crayfish by PCR (detection of crayfish plague carriers)

Birgit Oidtmann
Cefas, Weymouth Laboratory, Weymouth Dorset, UK

The currently recommended OIE method for diagnosing crayfish plague was presented. The primers used bind in the region of the internal transcribed spacer and amplify a 569 bp product. The specificity and sensitivity of the PCR has been tested: when using 50 cycles, the detection limit was determined as 1 spore of *Aphanomyces astaci* or 100 fg DNA. Another PCR protocol, using nested PCR, was presented that can be used if the DNA has potentially been damaged. This protocol uses the same primers as the previously mentioned PCR in the first round and a second primer in the nested round. This protocol may be useful if the samples have been fixed in formalin or ethanol or specimens arrive at the lab in autolytic condition. The advantages of PCR compared to culture are speed, the ability to use samples of a variety of conditions, including fixed material.

Detection of North American crayfish as carriers of crayfish plague uses the PCR methods mentioned above. Investigations on Signal crayfish (*Pacifastacus leniusculus*) and spiny cheek crayfish (*Orconectes limosus*) have shown that the best tissues to use are Telson and soft abdominal cuticle. Analysing both tissues in an animal increases the likelihood to identify an animal as detected by 30–40 %. Results from testing several populations of crayfish have revealed that there is a wide range of prevalence of infection, ranging from 0–100% in populations tested.

A TaqMan real-time PCR assay for specific and quantitative detection of *Aphanomyces astaci*

Trude Vrålstad, National Veterinary Institute (NVI), Oslo, Norway

Many limitations are connected to the diagnosis of crayfish plague based on classic approaches. Isolation of *A. astaci* in pure culture is highly specific if successful, but poses a considerable risk for false negatives. Direct microscopy commonly reveals a developed infection, but poses a risk for false positives (observation of oomycetes other than *A. astaci*) and false negatives (no observations despite presence of the agent). Provided the required specificity and caution, molecular detection represents a powerful tool for rapid and sensitive detection of *A. astaci* from a broad range of materials, including early infections, degraded remnants and historical samples. However, the reliability of the results depends heavily on the personnel handling of the samples during processing and analysis. Even though a molecular method has proved specific to *A. astaci*, positive results are reliable only if all steps during sample processing and preparation, DNA-isolation, PCR setup and post-PCR work have not introduced cross contaminations. In order to control for false positives during these steps, we always include environmental controls (open tube(s) with milliQ water following all working processes), DNA isolation controls (no-template tube(s) included in all steps of the DNA extraction) and negative PCR-controls. During sample preparation, we divide the samples (cuticle, eyes, telson, muscle etc) in two equal series of sub-samples annotated A and B. All controls are tested together with the A sub-samples. If positives are detected in any of the controls, the results will not be trusted or reported. In such cases, or if conclusions are difficult to draw based on the A-results alone, the B sub-samples (including new sets of controls) will processed and analyzed separately.

Conventional PCR rely on primers that in most cases allow PCR-amplification in the presence of a few mismatches. Additionally, gel control of PCR products and eventual confirmative sequencing are leading to the exposure of PCR products in the laboratory environment. Real-time PCR is monitoring the amplification of target DNA, and is therefore used both for detection and quantification purposes. Real-time PCR reactions are detected by fluorescence during the reaction (in "real-time"), and the products will never be exposed to the laboratory environment. The method is fast, cost efficient, and increasingly used and accepted for diagnostic purposes. To avoid the problems associated with detection of non-specific PCR amplicons, one or more fluorescent probes can be added to the reaction mix. The probe(s) are designed to be complementary to a sequence motif in the target amplicon, and a PCR product will only be detected if the amplified sequence is complementary to the annealing probe. TaqMan® assays utilize dually labeled probes with a reporter fluorophore covalently attached to one end of the oligonucleotide and a quencher attached to the other. When the probe anneals to the PCR product during the elongation step, the 5′ exonuclease activity of the polymerase cleaves the probe releasing the reporter dye, allowing it to move away from the quencher. As a result, the fluorescence of the reporter dye can be detected. To improve the specificity of such hydrolyzing probes, minor groove binding (MGB) probes have been developed. These are conjugated with a minor groove binder group that will increase the melting temperature (T_m) of the oligonucleotide, and hence improve the specificity of the hybridization. The increased T_m will also allow design of shorter probes, which

can be a great advantage in the design of species specific detection assays.

We developed a species specific TaqMan real-time PCR assay for quantitative detection of Aphanomyces astaci and compared this to other diagnostic methods. The designed TaqMan-MGB probe was based on a 13 bp ITS1 sequence motif that to all current knowledge is unique to *A. astaci*. The assay specificity was confirmed by BLAST searches as well as tests against closely related Oomycetes. No cross reactions were observed with the closest relatives, including *A. invadans*, *A. piscicida* and *A. frigidophilus*. A four-fold dilution series with genomic *A. astaci* DNA was used to establish a standard curve for quantification. The limit of detection (LOD) and limit of quantification (LOQ) was estimated to ~1 and ~125 PCR forming units (PFU) of the multi-copied ITS rDNA, corresponding to ~ 0.01 and 1 genome, respectively. A total of 57 individuals of Astacus astacus representing 21 cases of suspected crayfish plague in Norway (2005) were tested. The RT-PCR assay proved highly specific and sensitive, and detected *A. astaci* in 17 of the 21 cases. Two other molecular assays based on conventional PCR and sequencing supported the detection of *A. astaci* in 14 of these cases, while direct microscopy supported classic infection of oomycete hyphae in nine cases. In contrast, *A. astaci* was only isolated in pure culture once. The real-time assay is presently used as the "standard" crayfish plague detection method in Norway, and has also been used and proved suitable for detection of *A. astaci* in symptom free carrier crayfish. We also expect that the method may be suitable for direct detection in water and environmental samples. Future perspectives include among others further validation of the method, preferably through a collaborative trial.

Crayfish disease research at the Kuopio Unit, Finnish Food Safety Authority Evira

Satu Viljamaa-Dirks and Hannu Torssonen,
Evira Kuopio, Finland

The duties of the Finnish Food Safety Authority Evira include scientific research, risk assessment and diagnostic studies of animal diseases. The Authority also operates as a reference laboratory in its own field. The Kuopio unit works in the field of animal health, providing services in animal disease diagnostics as well as animal health care. The laboratory is well equipped to perform pathological and microbiological analyses, including molecular biology. The Kuopio unit is responsible for the animal disease diagnostics in Eastern and Central Finland. Disease diagnostics concerning crayfish and honey bees cover the whole country. We also have facilities to perform experimental work with aquatic animal diseases.

One of the research topics of the Kuopio unit is diagnostics and epidemiology of crayfish plague. The laboratory studies several hundred crayfish field specimens every year. Epidemiological information about disease outbreaks is gathered as well. We maintain a unique collection of *Aphanomyces astaci* isolates, and several isolates are added yearly.

During 2006-2007 we undertook a research project in co-operation with The Finnish Game and Fisheries Research Institute and Kuopio University, funded by European Union. The aim of this project was to study the epidemiology of crayfish plague and to improve the diagnostics, especially in situations with low-level infection.

Epidemiological information gained in connection with clinical samples point to variable virulence between the genotypes. We performed an infection trial using noble crayfish as susceptible host. Two PsI-type strains and two As-type strains were chosen, based on the radial growth rate differences. Significant differences were seen in the development of mortality in test groups. The representatives of PsI-type caused total mortality in the expected time span. As-type strains were found to be much less virulent. Although total mortality was achieved in this trial, it took more than half a year to develop with a less virulent strain. It can not be excluded, that in natural circumstances, where the infection dose can be much lower and the water temperature less favourable for the growth of *A. astaci*, a low-virulent strain, even carried by a susceptible host, can survive for prolonged periods.

Crayfish research at University of Kuopio

Kokko Harri, Makkonen Jenny, Jussila Japo, Henttonen Paula, Alaranta Anna, Halmekytö Marja and Atte von Wright
Department of Biosciences, University of Kuopio, P.O.Box 1627, FIN-70211 Kuopio
Finland Crayfish Innovation Centre, Meijeritie 1, FIN-17200 Vääksy

Crayfish Team includes University of Kuopio (UKU), Department of Biosciences staff and students (4 academic staff + 4 under and postgraduate students) and Crayfish Innovation Centre (CIC) staff (2 craybiologists). With this small network collaboration we are covering research & development, application of results and education of both academic students and laypersons. We are focusing on two main topics: the genetic differences among noble crayfish stocks and crayfish plague strains in Finland and also neighbouring countries. There are currently running two PhD–projects and several undergraduate projects studying different aspects of these two topics. We are participating in a joint project on crayfish plague (*Aphanomyces astaci*) diagnostics and understanding of differences among crayfish plague strains in wild populations. We also have close connections to crayfish farming industry and to persons involved in wild stock management and exploitation. Key targets of current research & development: We are also focusing on implementation of the results to practical, hands-on situations. Crayfish Innovation Centre is involved in several local crayfisheries projects were local fishermen are educated on how to best manage wild and farmed crayfish stocks, with special emphasis on the prevention of the spread of diseases and sustainable exploitation of wild stocks. Current UKU crayfish team networking: Crayfish Innovation Centre is currently active partner in UKU PhD training and research & development and CIC acts as a platform for contacts to crayfish farming and wild stock management for UKU. This consortium has a wide network covering Scandinavia (Freshwater laboratory in Drottningholm, Uppsala University and National Veterinary Institute in Oslo), Estonia (Tarto University) and also European crayfish institutions (CRAYNET and personal contacts) and wider including Australia, via personal contacts and IAA.

Annex I
Programme

"CRAYFISH DISEASE DIAGNOSTICS- TOWARDS A NORDIC STANDARD"

Scandinavian workshop 7. - 8.2.2007
Finnish Food Safety Authority Evira, Kuopio Unit,
Neulaniementie 4, Kuopio, Finland

Wednesday 7.2.	**"Crayfish disease- confirmation and consequences"**	
	10.00-10.30	Crayfish in Finland Timo Ruokonen, Markku Pursiainen, Riitta Savolainen Finnish Game and Fisheries Research Institute
	10.30-11.00	Crayfish disease in Finland Satu Viljamaa-Dirks, Finnish Food Safety Authority
	11.00-11.30	Crayfish and crayfish disease in Estonia Tiit Paaver, Estonian Agricultural University
	11.30-12.00	Crayfish and crayfish disease in Central Europe Birgit Oidtmann, CEFAS
	12.00-13.00	Lunch
	13.00-14.00	Crayfish in Norway Stein Ivar Johnsen , Norwegian Institute for Nature Research Crayfish plague in Norway - outbreaks and diagnosis Trude Vrålstad, National Veterinary Institute, Norway
	14.00-15.00	Crayfish and crayfish disease in Sweden Thorbjörn Hongslö, Eva Jansson, SVA
	15.00-15.30	Coffee
	15.30-15.50	Crayfish and crayfish disease in Latvia Antra Skutela, Rita Granta, National Diagnostic Centre
	15.50-16.20	Crayfish and crayfish disease in Lithuania Aloyzas Burba, Liongina Mickeniene, Vilnius University
	16.20-17.00	OIE- listing of crayfish plague Discussion over possibilities and problems
Thursday 8.2.	**"Research in crayfish disease diagnostics"**	
	9.00-10.00	Molecular methods in crayfish plague diagnostics Birgit Oidtmann, Trude Vrålstad
	10.00-11.00	Crayfish disease research projects Evira: Satu Viljamaa-Dirks, Hannu Torssonen Crayfish studies in University of Kuopio: Harri Kokko, Jenni Makkonen Finnish Game and Fisheries Research Institute
	11.00-12.00	Crayfish disease diagnostic network, future co-operation. Discussion
	12.00-13.00	Lunch
	13.00-	Project planning in groups (optional)

Annex II
List of participants

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